Vacuum UV Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry with ice as matrix

C500 Report

Yi He

Research Advisor: Dr. James Reilly

Department of Chemistry

Indiana University

April 22, 2005

I. Introduction

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF) is becoming a standard tool in mass spectrometry in protein analysis. Its advantages include fast speed of data acquisition, high sensitivity, tolerance to buffers and simple instrumentation[1].

1. Matrices

The matrix is an important role on energy absorption and analyte isolation during the MALDI process. The selection of a MALDI matrix is critical: Firstly, a suitable MALDI matrix must be soluble in solution with the analyte but chemically inert with the analyte. Secondly, the MALDI matrix should have a strong absorption coefficient at the wavelength of the laser. Research has concentrated on the near-UV region, limiting the choice of matrices to aromatic compounds with electron-withdrawing groups. Furthermore, potential matrix compounds must also have low sublimation rates, since the sample must be introduced into vacuum for mass spectrometric analysis. High sublimation rates would result in significant changes in the matrix-to-analyte ratio during an experiment, thus affecting the results.

In the sample preparation procedure, the analyte is typically dissolved in aqueous solution. As the solvent evaporates, matrix crystals are formed in several places and become easily visible by the naked eye.

A large variety of compounds has been tested in the past for their suitability to act as a matrix. Derivatives of benzoic acid, cinnamic acid, and related aromatic compounds were recognized early as good MALDI matrices for proteins (Beavis & Chait, 1989).

2. Laser wavelength

The laser wavelength is a particularly important parameter in MALDI. By far in most cases, lasers with wavelengths in the near-ultraviolet are today employed for MALDI. N2 lasers (wavelength = 337 nm) and frequency-tripled Nd:YAG lasers (wavelength = 355 nm) are commonly used. Pulse durations of these lasers are typically in the range of 0.5 to ~10 ns[3].

Table 1 gives the wavelengths and the corresponding photon energies for these laser sources; typical pulse widths are also included[2]. There are still other laser sources whose use remains limited to specialized research purposes, for example tunable lasers, such as dye lasers, optical parametric oscillators, and free electron lasers.

Table 1. Laser wavelengths, pulse widths, and corresponding photon energies.

Laser	Wavelength	Photon energy (kcal/mol)	Photon energy (eV)	Pulse width
Nitrogen	337 nm	85	3.68	<1 ns – few ns
Nd:YAG × 3	355 nm	80	3.49	typ. 5 ns
Nd:YAG × 4	266 nm	107	4.66	typ. 5 ns
Excimer (XeCl)	308 nm	93	4.02	typ. 25 ns
Excimer (KrF)	248 nm	115	5.00	typ. 25 ns
Excimer (ArF)	193 nm	148	6.42	typ. 15 ns
Er:YAG	2.94 μm	9.7	0.42	85 ns
CO_2	10.6 μm	2.7	0.12	100 ns + 1 μs tail

In our experiment, ice is used as a new type of matrix coupled with vacuum UV, since most proteins can be dissolved in water and ice has a strong absorption in the range of 100~200 nm as showed in Figure 2[9]. Ice may be a good matrix for the mass spectrometry for proteins. Thus sample-matrix preparation becomes much easier and there is a good chance to see protein complexes in mass spectrum.

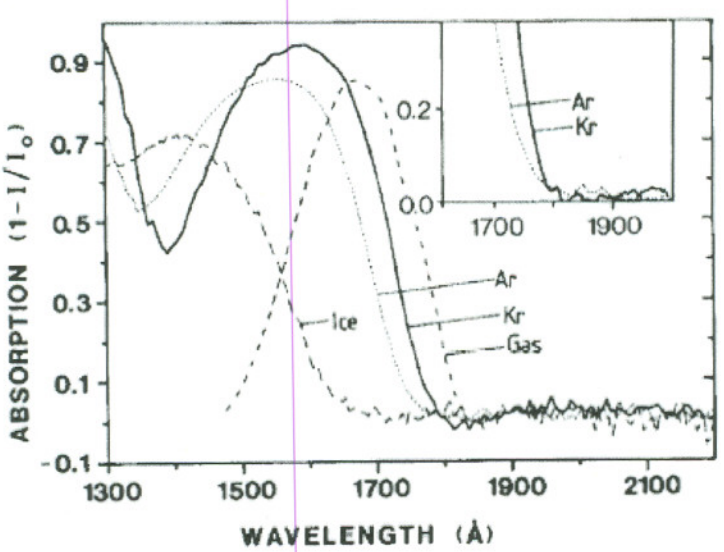

Figure 2. Absorption spectrum of 1 Torr H_2O vapor, of ice (sample thickness ~0.02 μm) and of 0.2% H_2O in Ar and Kr matrices (sample thickness ~~25 pm). The insert shows the absorption in the threshold region for o 100 μm thick Ar and Kr samples.

Another thing we are interested in is an unusual set of peaks observed by Randy J. Arnold in previous work[5, 6]. Those peaks were all evenly spaced by approximately 129Da at the mass range of 1-2kDa and 3-4kDa. The explanation for those peaks is that several glutamates would be attached to folic acid. It is generally agreed that attaching polyglutamate to folic acid increases its retention in cells and makes the folic acid a better substrate and cofactor. However, it is really unusual to see 20 or 30 glutamates being attached to folic acid (the peaks between 3 and 4kDa). In this research work, we try to reproduce Randy Arnold's polyglutamate results by using a home-built linear time-of-flight mass spectrometer and it's also a practice to gain familiarity with the instrument.

II. Experimental

1. Test of the equipment

Experiments were carried out on a home built pulsed-ion extraction linear time-of-flight mass spectrometer as shown in Figure 3. To improve the resolution of the mass spectrometer, 6 proteins mixture solution containing myoglobin (0.1mM), hemoglobin (0.1mM), lysozyme (0.1mM), cycchrom C (0.1mM), ribonuclease A (0.1mM) and carbonic anhydrase II (0.1mM) was tested by varying the accelerating voltage and the delay extraction.

2. Study of E. coli.

Cell growth and extraction

All cultures used in this study were grown in house at 37℃ shaking at 200 rpm. They were inoculated from a starter culture of K-12 Escherichia coli (ATCC 25404, obtained from the Indiana University Biology Department) that had been grown the previous night. Cultures were inoculated with 15 μL of this starter culture. E. coli. were grown aerobically in a 1000-mL Erlenmeyer flask filled with 200 mL of Luria Bertani (LB) medium(1% tryptone, 0.5% yeast extract, 1% sodium chloride, PH=8). Duplicate 1-mL aliquots were sampled from culture at 2-h intervals from 32 to 50 h after inoculation. Cells were harvested by centrifugation (7000g for 5 min), resuspending the pellets with 0.5ml of a 50mM pH=8.0 Tris buffer containing 50 mM glucose and 10 mM EDTA in order to inhibit their growth and keep them intact, centrifuging at 7000g for 5 minutes again, and decanting the supernatant. The

resulting pellet was stored dry at -20℃ for at least 12 h before analysis. At harvest, the optical density (OD) at 600 nm was measured for each culture a Hitachi 300 spectrophotometer.

MALDI sample preparation

The pellet of frozen cells were thawed and diluted with distilled water. In order to ensure that this suspension of cells had a consistent concentration for the different sample, the pellets were diluted with a volume proportional to the optical dentsity of the original cell culture. An optimal concentration was found when diluting the pellet with 40μL of water per 1 o.d. (600nm) of the original culture. MALDI spots were made by mixing 1 μL of bacterial suspension with 9 μL of a 10 mg/mL solution of R-cyano-4-hydroxycinnamic acid (CHCA, Aldrich Chemical) dissolved in 2 parts 0.1% trifluoroacetic acid (TFA, obtained from Fisher Scientific) and 1 part acetonitrile (Fisher Scientific). A One microliter anliquot of this solution was deposited onto a stainless steel probe and allowed to air-dry.

Spectra acquisition

Mass spectra were acquired with a pulsed-ion extraction linear time-of-flight mass spectrometer built in lab. MALDI spots were irradiated with 355-nm light from a frequency-tripled Nd:YAG laser. Positive ions were accelerated through 10 kV toward a dual microchannel plate detector. Spectra were recorded on a Lecroy model 9370 digital oscilloscope at a sampling rate of 500 MHz and signal averaged for 50 shots.

III Results and Discussion

1. 6 proteins mixture

In our experiment, different conditions (laser energy, delay extraction and accelerating voltage) have been varied to improve the resolution from 200 to 800. A series of results shown in Figure 4 is the mass spectrums of the mixture obtained at different accelerating voltage. The big broad peaks at very beginning of spectra are due to turning on the detector. From the first spectrum, only two components of the six proteins could be identified, and the peaks of proteins with double charges are difficult to see. Five proteins could be identified and some peaks of double charged protein can be identified in the last spectrum. Due to the improvement of resolution, proteins can easily be distinguished from those adducts of matrix molecules to the protein molecule.

2. Study of E. coli.

In this research, we reproduce the evenly spaced peaks at low mass range 1-2kDa shown in Figure 5-11, but the peaks around 3-4kDa haven't been observed from these spectra.

The even spaced peaks that appear around 1500 Da correspond to 5-6 glutamates being attached to folic acid and could be identified clearly after 40h of inoculation. The signal intensity is increasing with the length of growing period.

IV Future work

Ice will be used as matrix in the study of bacteria coupled with vacuum UV in the following research work. Experimental conditions (laser wavelength, laser energy, delay extraction and accelerating voltage) will be varied to find the best parameter for ice matrix. The results will be compared (sensitivity, resolution) with those using R-cyano-4-hydroxycinnamic acid (CHCA) as matrix to find whether ice could be suitable for mass spectrometry of proteins.

References:

[1] Ole Vorm, Peter Roepstorff,t et al. *Analytical Chemistry*, 1994, 66, 3281-3287

[2] Renato Zenobi, Richard Knochenmuss. *Mass Spectrometry Reviews*, 1998, 17, 337-366

[3] Klaus Dreisewerd. *Chem. Rev.* 2003, 103, 395-425

[4] Christopher.T. Houston. PHD thesis, Department of chemistry, Indiana University, 1999

[5] Randy J Arnold and James P. Reilly. *Analytical Biochemistry*, 2000, 281, 45-54

[6] Randy J. Arnold, Jonathan A. Karty, Andrew D. Ellinton, and James P. Reilly. *Anal. Chem.* 1999, 71, 1990-1996

[7] Marcus Macht, Arndt Asperger and So¨ ren-Oliver Deininger. Rapid Commun. Mass Spectrom, 2004, 18, 2093–2105

[8] Michael Karas* and Ralf Kru¨ger. *Chem. Rev.* 2003, 103, 427-439

[9] M. Chergui, N. Schwentner. Chemical Physics Letters 1994, 219, 237-242

[10] Michael Karas,* Doris Bachmann, and Franz Hillenkamp。 Anal. Chem. 1985, 57, 2935-2939

[11] Ronald C. Beavis and Brian T. Chait. Rapid Commun. Mass Spectrom, 1989, 3, 432–435

[12] K. Strupat, M. Karas and F. Hillenkamp. Int. J. Mass Spectrom. Ion Proc., 1991, 111, 89-102

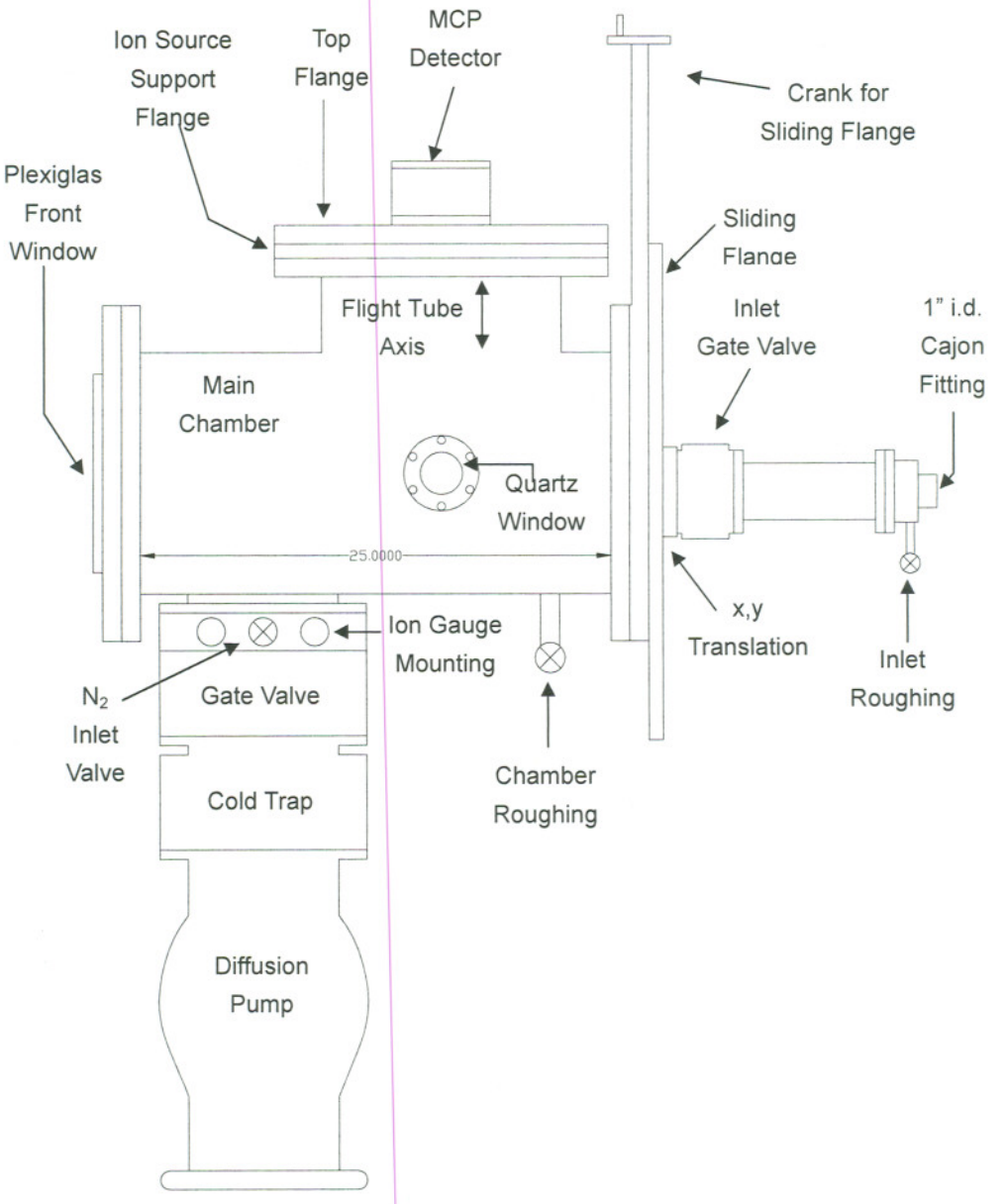

Figure 3. Exterior view of MALDI time-of-flight mass spectrometer.

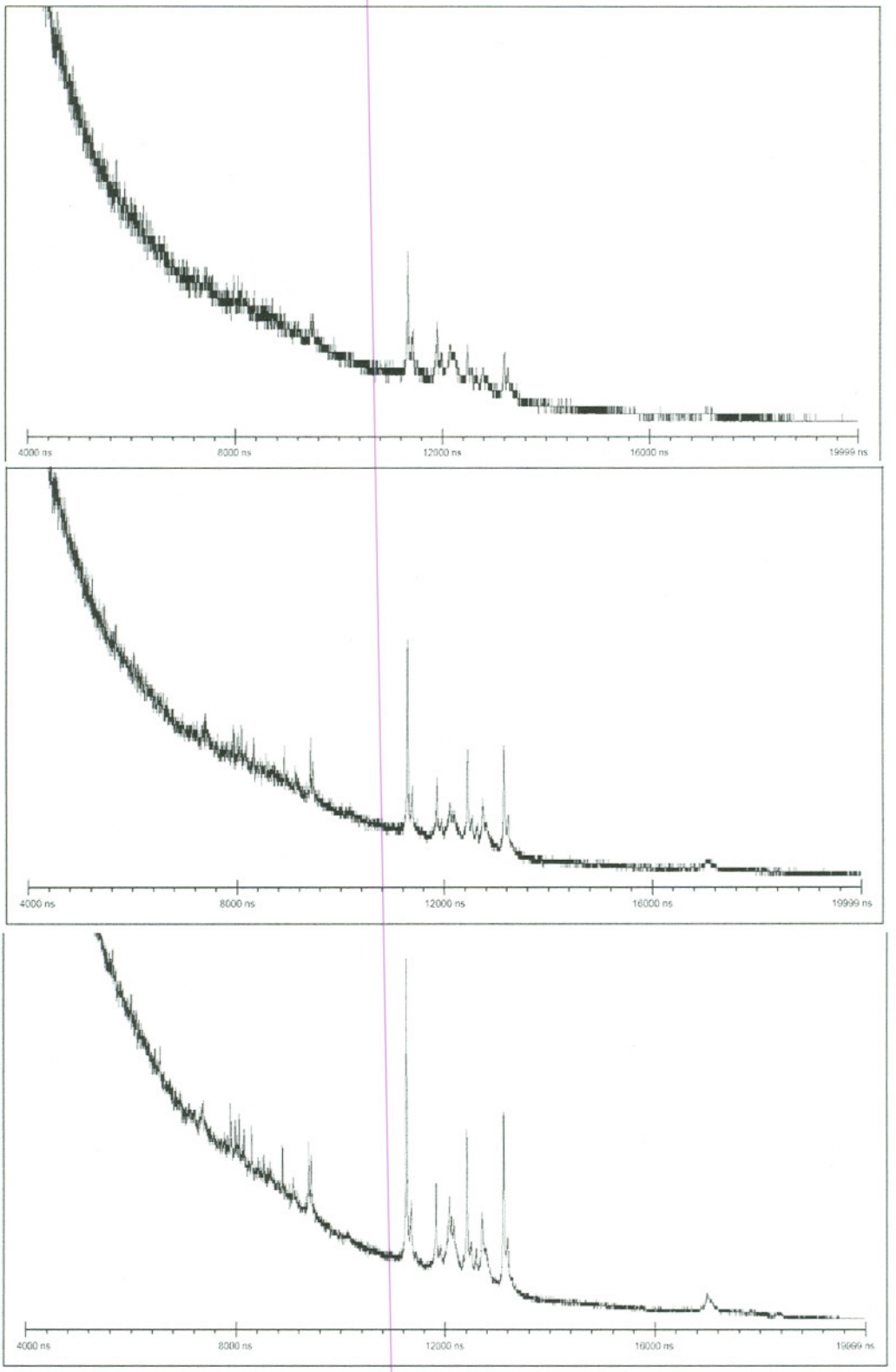

Figure 4. spectrums of 6 proteins mixture by varying accelerating voltage

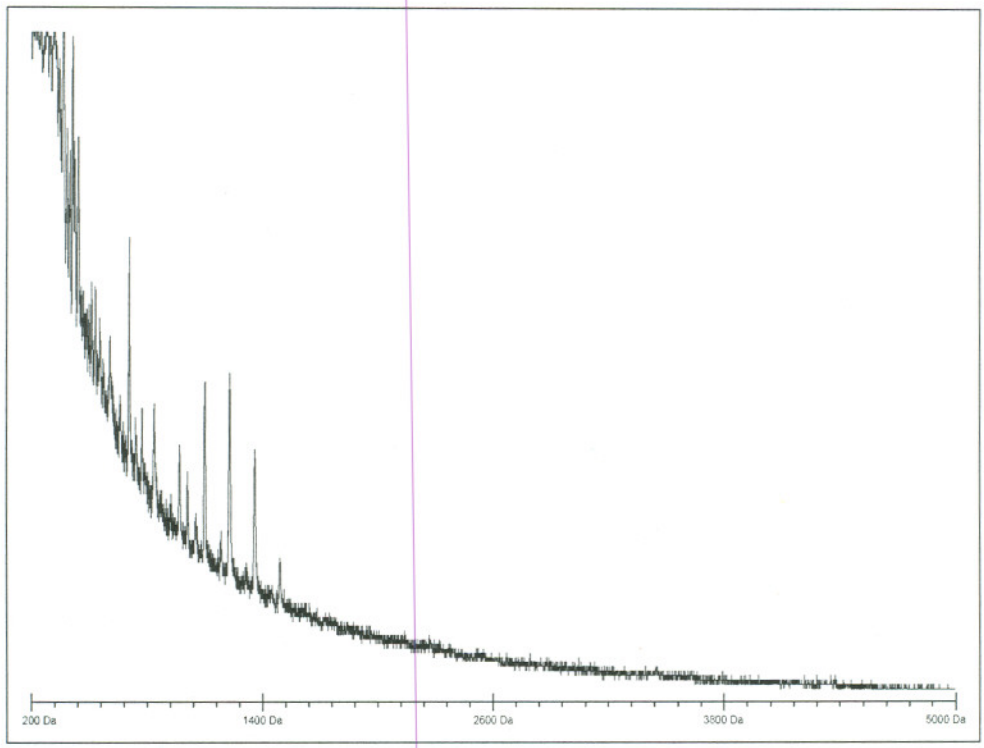

Figure 5. Mass spectrum of E. coli strain K-12, sampled at 32h after inoculation.

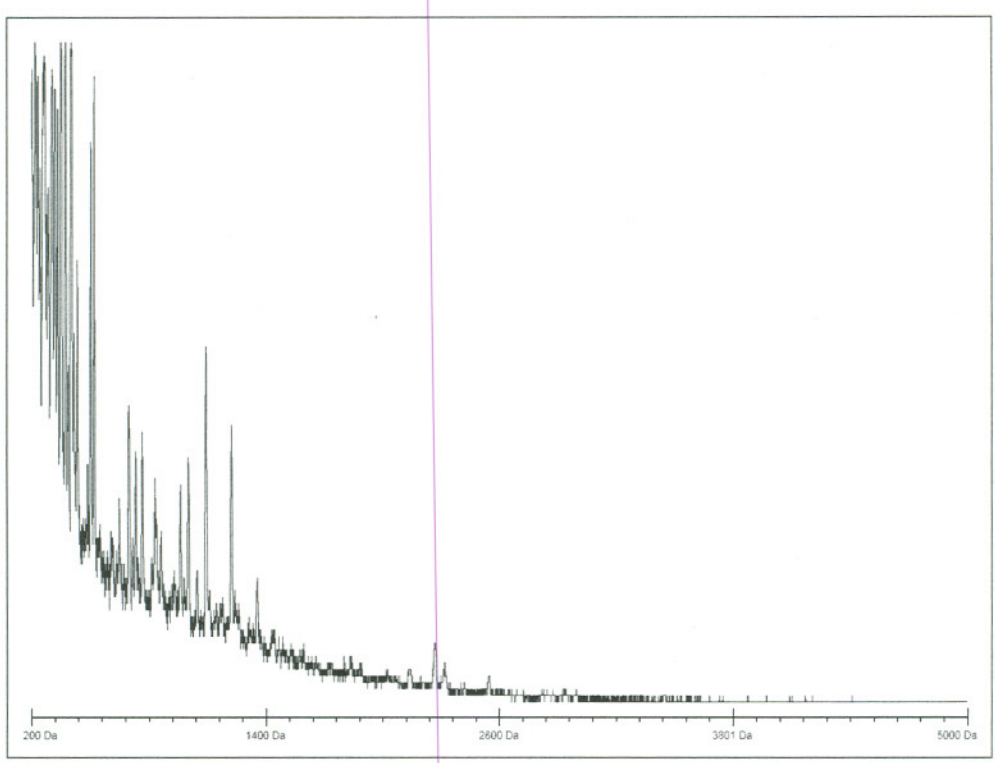

Figure 6. Mass spectrum of E. coli strain K-12, sampled at 36h after inoculation.

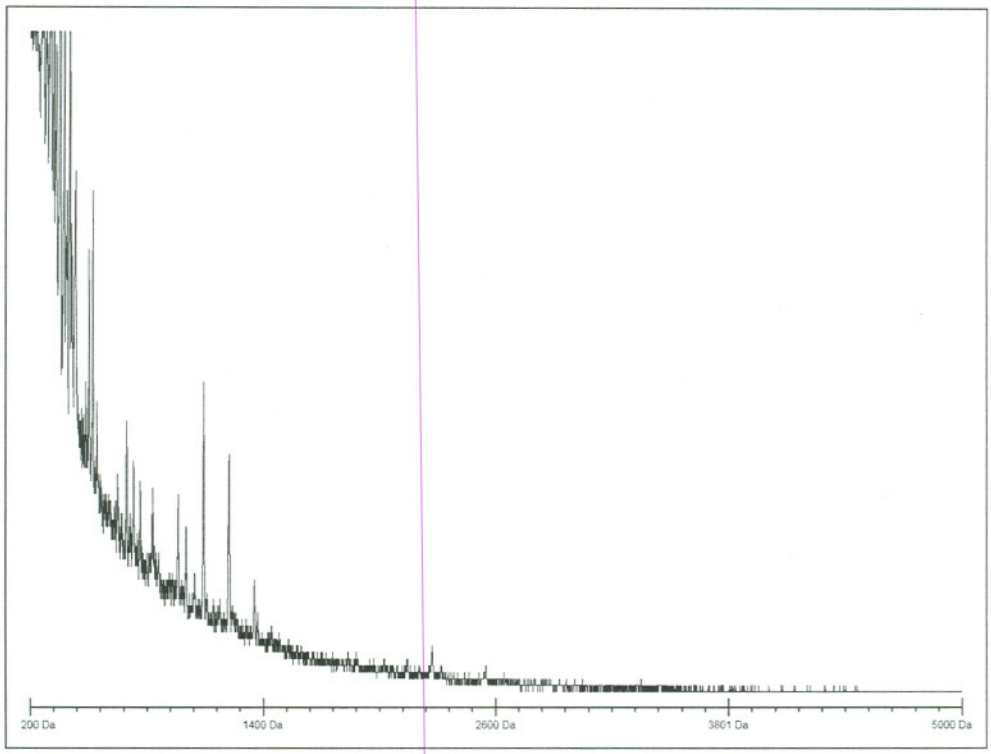

Figure 7. Mass spectrum of E. coli strain K-12, sampled at 40h after inoculation.

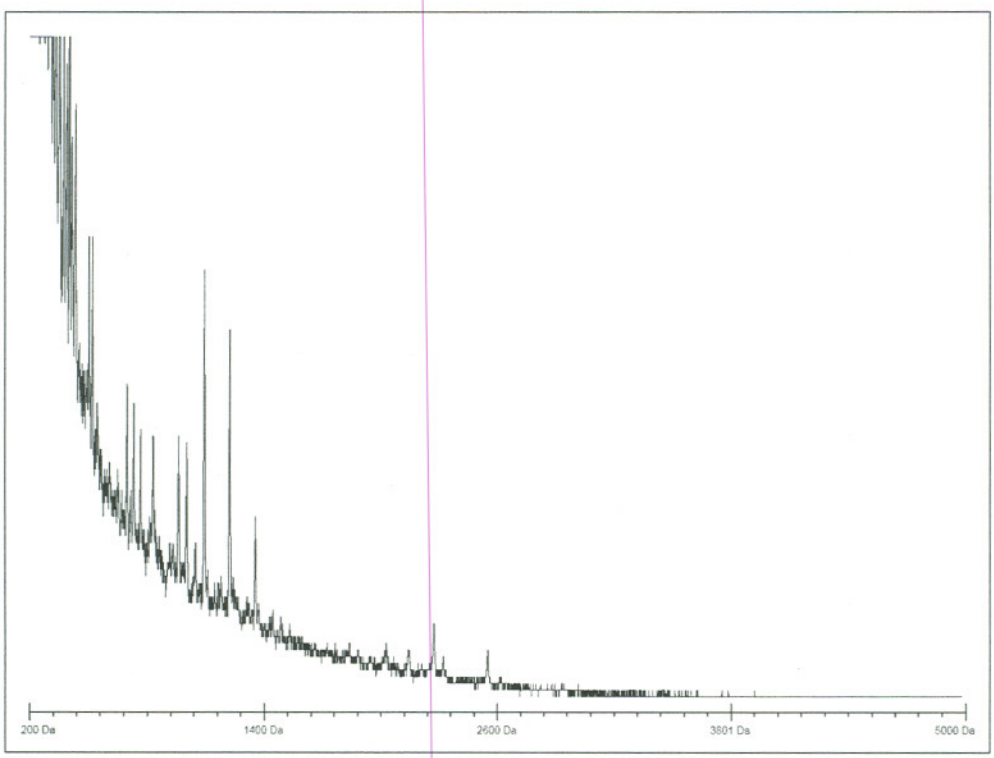

Figure 8. Mass spectrum of E. coli strain K-12, sampled at h 42h after inoculation.

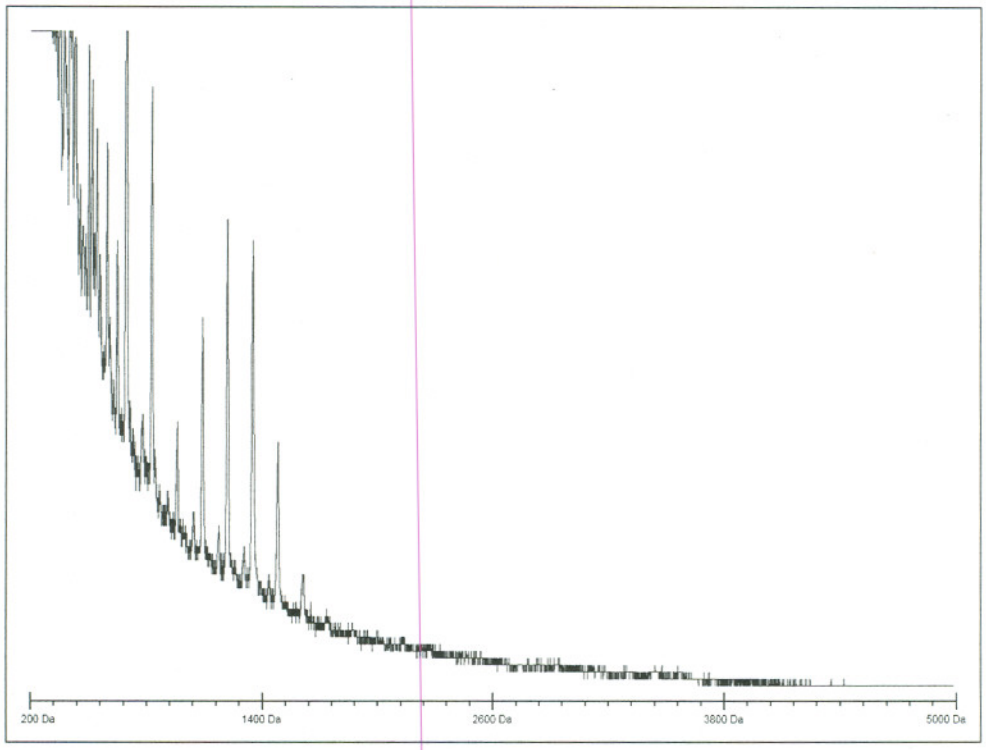

Figure 9. Mass spectrum of E. coli strain K-12, sampled at 44h after inoculation.

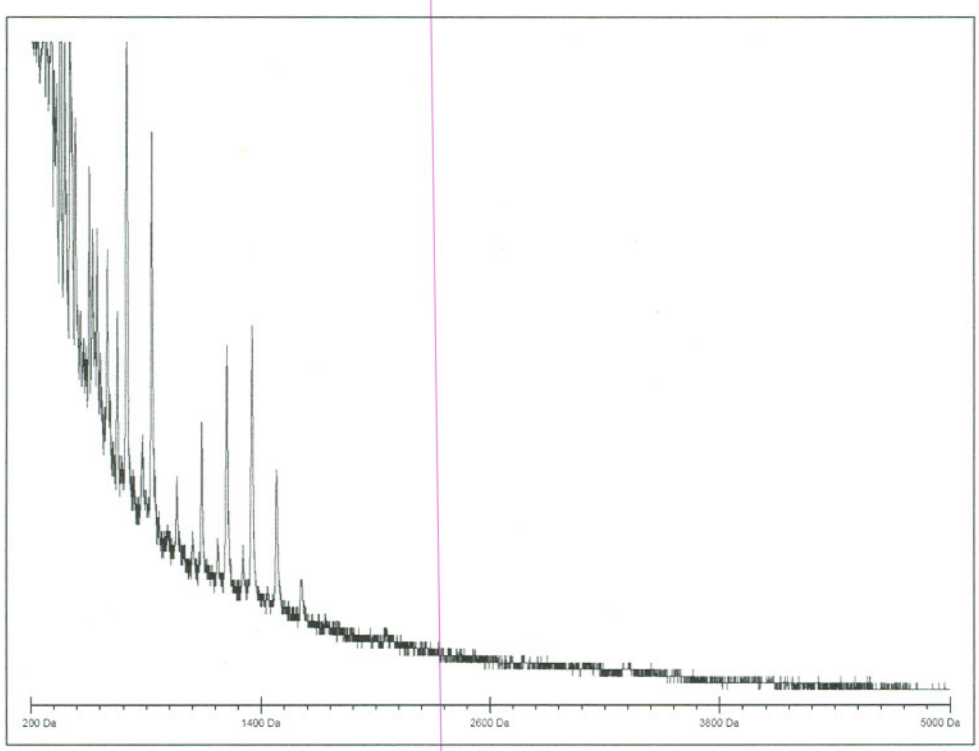

Figure 10. Mass spectrum of E. coli strain K-12, sampled at 48h after inoculation.

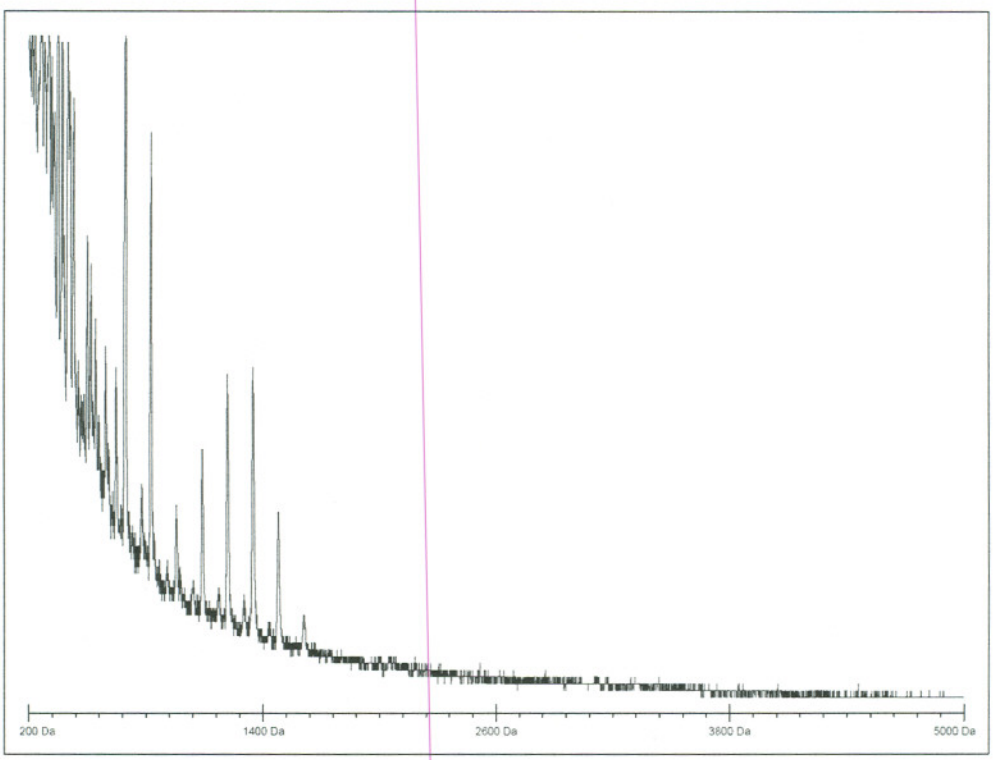

Figure 11. Mass spectrum of E. coli strain K-12, sampled at 50h after inoculation.